（FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS !）

Catalog No：E-BC-K823-M

Specification：96T(40 samples)

Measuring instrument：Microplate reader (630-640 nm)

Detection range：0.216-7.200 U/L

Elabscience® Protein Tyrosine Phosphatase-1B (PTP-1B) Activity Colorimetric Assay Kit

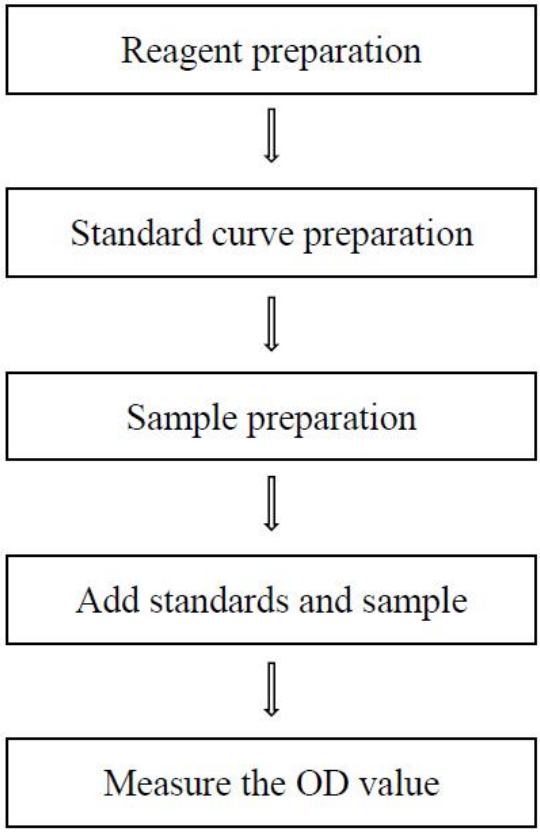
This manual must be read attentively and completely before using this product.
If you have any problem, please contact our Technical Service Center for help：

Toll-free: 1-888-852-8623
Tell: 1-832-243-6086
Fax: 1-832-243-6017
Email: techsupport@elabscience.com
Website: www.elabscience.com

Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

Table of contents

Assay summary



Intended use

This kit can be used to measure protein tyrosine phosphatase-1B (PTP-1B) activity in plant, animal tissue and cell samples.

Detection principle

Protein Tyrosine Phosphatase-1B (PTP-1B) belongs to the family of protein tyrosine phosphatases, which specifically hydrolyze aromatic phosphates and negatively regulate insulin signal transduction by dephosphorylating tyrosine residues on insulin receptors or their substrates.

In the dephosphorylation of PTP-1B, the phosphate group produced reacts with malachite green to form chromogenic substance, PTP-1B in samples is measured by measuring the absorbance change at 636 nm.

Kit components & storage

Item	Component	Size (96 T)	Storage
Reagent 1	Buffer Solution	55 mL × 1 vial	-20℃,12months
Reagent 2	Reducing Reagent	5 mL × 1 vial	-20℃, 12 months, shading light
Reagent 3	Substrate	Power × 2 vials	-20℃, 12 months, shading light
Reagent 4	Chromogenic Agent A	24 mL × 1 vial	-20℃, 12 months, shading light
Reagent 5	Chromogenic Agent B	8 mL × 1 vial	-20℃, 12 months
Reagent 6	Protein Precipitator	60 mL × 1 vial	-20℃, 12 months
Reagent 7	1 mmol/L Standard Solution	1 mL × 1 vial	-20℃, 12 months, shading light
	Microplate	96 wells	No requirement
	Plate Sealer	2 pieces	

Note: The reagents must be stored strictly according to the preservation conditions in the above table. The reagents in different kits cannot be mixed with each other. For a small volume of reagents, please centrifuge before use, so as not to obtain sufficient amount of reagents.

Materials prepared by users

Instruments:

Microplate reader (630-640 nm, optimum wavelength: 636 nm), Incubator

Reagent preparation

① Equilibrate all reagents to 25℃ before use.

② The preparation of extraction solution：

Before testing, please prepare sufficient extraction solution according to the test wells. For example, prepare 500 μL of extraction solution (mix well 450 μL of buffer solution and 50 μL of reducing reagent) and protected from light for testing. Store at 2-8°C for 3 days.

③ The preparation of reaction working solution：

Dissolve one vial of substrate with 3 mL of double distilled water, mix well to dissolve. Store at -20°C for 10 days protected from light.

④ The preparation of chromogenic working solution：

Before testing, please prepare sufficient chromogenic working solution according to the test wells. For example, prepare 800 μL of chromogenic working solution (mix well 300 μL of chromogenic agent A, 100 μL of chromogenic agent B and 400 μL of double distilled water). Incubate the prepared chromogenic working solution at 37°C for 1 h, keep it at 25℃ protected from light, and use up within 1 day (chromogenic agent A may have gelatinous substances, please warm it in 90℃ water-bath until clear).

⑤ The preparation of 200 μmol/L standard solution：

Before testing, please prepare sufficient 200 μmol/L standard solution. For example, prepare 1000 μL of 200 μmol/L standard solution (mix well 200 μL of 1 mmol/L standard solution and 800 μL of double distilled water). Store at -20°C for a month.

⑥ The preparation of standard curve：

Always prepare a fresh set of standards. Discard working standard dilutions after use.

Dilute 200 μmol/L standard solution with double distilled water to a serial concentration. The recommended dilution gradient is as follows: 0, 40, 60, 80,

100, 120, 140, 200 μmol/L. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (μmol/L)	**0**	**40**	**60**	**80**	**100**	**120**	**140**	**200**
200 μmol/L standard (μL)	0	40	60	80	100	120	140	200
Double Distilled Water (μL)	200	160	140	120	100	80	60	0

Sample preparation

① Sample preparation

Tissue sample:

① Harvest the amount of tissue needed for each assay (initial recommendation 20 mg).

② Wash tissue in Wash tissue in normal saline (0.9% NaCl).

③ Homogenize 20 mg tissue in 180 μL extraction solution with a dounce homogenizer at 4℃.

④ Centrifuge at 10000×g for 10 min at 4℃ to remove insoluble material. Collect supernatant and keep it on ice for detection. Detect the prepared sample within 4 h.

Cell sample

① Harvest the number of cells needed for each assay (initial recommendation 1×10^6 cells).

② Wash cells with normal saline (0.9% NaCl).

③ Homogenize 1×10^6 cells in 200 μL extraction solution with a ultrasonic cell disruptor at 4℃.

④ Centrifuge at 10000×g for 10 min at 4℃ to remove insoluble material. Collect supernatant and keep it on ice for detection. Detect the prepared sample within 4 h.

② Dilution of sample

The recommended dilution factor for different samples is as follows (for reference only):

Sample type	Dilution factor
10% Mouse liver tissue homogenate	1
10% Mouse kidney tissue homogenate	1
10% Rat lung tissue homogenate	1
10% Mouse brain tissue homogenate	1
10% Green radish tissue homogenate	1
1×10^6 Jurkat cells	1

Note: The diluent is extraction solution. For the dilution of other sample types, please do pretest to confirm the dilution factor

The key points of the assay

① Operate carefully during the experiment, and the experimental equipment used should be washed many times (about 10 times) to avoid external phosphorus pollution.

② If the OD value of the sample well is greater than 1.30, the supernatant of the incubation reaction step will be diluted with protein precipitator in proportion, and then the chromogenic reaction will be carried out.

③ During the operation procedure, when the supernatant collected in the fourth step of the incubation reaction is added to the microplate, it should be carefully absorbed to avoid precipitation.

④ PTP-1B activity will decrease with time, and it is recommended to measure it within 4 h after sample homogenization.

Operating steps

Incubation reaction

① Sample tube: add 20 μL of sample into the 1.5 mL EP tubes.
Control tube: add 20 μL of sample into the 1.5 mL EP tubes.

② Add 100 μL of reaction working solution into the sample tubes and add 100 μL buffer solution into the control tubes.

③ Incubate at 37℃ for 1 h.

④ Add 600 μL of protein precipitator into the tubes, centrifuge at 10000×g for 10 min. Collect supernatant and keep it at 25℃ for detection.

Chromogenic reaction

① Standard well: add 20 μL of different concentrations of standard solutions into the wells.
Sample well: add 20 μL of supernatant in the sample tubes into the wells.
Control well: add 20 μL of supernatant in the control tubes into the wells.

② Add 200 μL of chromogenic working solution into the wells.

③ Mix fully with microplate reader for 5 s. Incubate at 37°C for 20 min, and measure the OD value of each well at 636 nm with microplate reader.

Calculation

The standard curve:

1. Average the duplicate reading for each standard.

2. Subtract the mean OD value of the blank (Standard #①) from all standard readings. This is the absoluted OD value.

3. Plot the standard curve by using absoluted OD value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve (**y = ax + b**) with graph software (or EXCEL).

The sample:

1. Tissue sample:

Definition: The amount of enzyme in 1 kg tissue of 1 L reaction system per 1 h that produce 1 mmol phosphate at 37℃ is defined as 1 unit.

$$\text{PTP-1B activity (U/kg wet weight)} = (\Delta A_{636} - b) \div a \times \frac{V_1}{V_2} \div \frac{m}{V_3} \times f \div T \div 1000^*$$

2. Cell sample:

Definition: The amount of enzyme in 10^6 cell of 1 L reaction system per 1 h that produce 1 μmol phosphate at 37℃ is defined as 1 unit.

$$\text{PTP-1B activity (U/10^6)} = (\Delta A_{636} - b) \div a \times \frac{V_1}{V_2} \div \frac{n}{V_3} \times f \div T \div 1000^*$$

[Note]

ΔA_{636}: Absolute OD value, $OD_{Sample} - OD_{Control}$.

f: Dilution factor of sample before test.

m: The wet weight of tissue, g.

n: The number of cell samples, 10^6.

V_1: The total volume of incubation reaction, 0.72 mL.

V_2: The volume of sample to be measured, 0.02 mL.

V_3: The volume of extraction solution, mL

T: Reaction time, 1 h.

1000*： 1 mmol/L=1000 μmol/L

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three mouse liver samples were assayed in replicates of 20 to determine precision within an assay (CV = Coefficient of Variation).

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	3.40	2.40	1.60
%CV	2.0	2.1	2.0

Inter-assay Precision

Three mouse liver samples were assayed 20 times in duplicate by three operators to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	3.40	2.40	1.60
%CV	7.5	10.0	8.3

Recovery

Take three samples of high concentration, middle concentration and low concentration to test the samples of each concentration for 6 times parallelly to get the average recovery rate of 101%.

	Sample 1	Sample 2	Sample 3
Expected Conc. (U/L)	3.4	2.4	1.6
Observed Conc. (U/L)	3.2	2.6	1.6
Recovery rate (%)	95	107	101

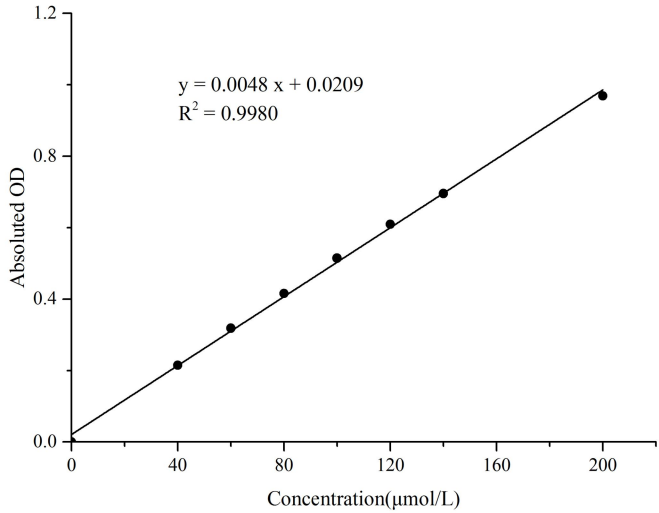
Sensitivity

The analytical sensitivity of the assay is 0.216 U/L. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

2. Standard curve:

As the OD value of the standard curve may vary according to the conditions of the actual assay performance (e.g. operator, pipetting technique or temperature effects), so the standard curve and data are provided as below for reference only:

Concentration (μmol/L)	0	40	60	80	100	120	140	200
OD	0.305	0.524	0.632	0.729	0.829	0.919	1.006	1.279
Average OD	0.308	0.519	0.618	0.715	0.813	0.913	0.998	1.272
	0.307	0.522	0.625	0.722	0.821	0.916	1.002	1.276
Absoluted OD	0	0.215	0.319	0.416	0.515	0.610	0.696	0.969



Appendix II Example Analysis

Example analysis:

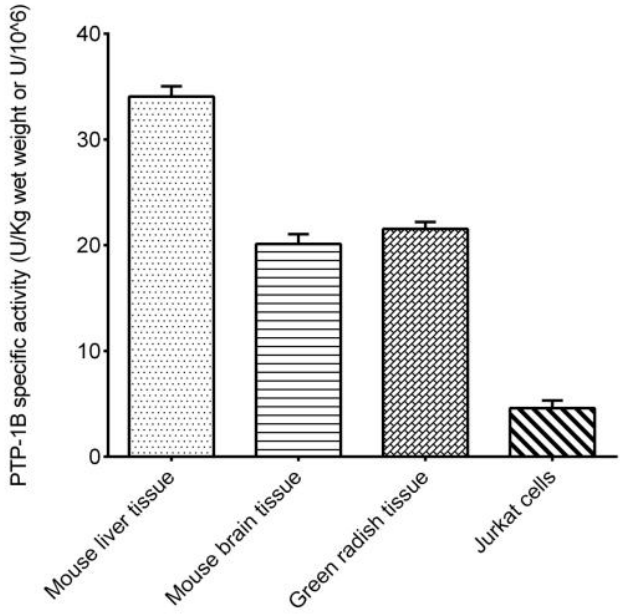
Take 20 μL of 10% mouse liver tissue homogenate into the well, and carry the assay according to the operation steps. The results are as follows:

The standard curve is y = 0.0048 x + 0.0209, the OD value of the sample well is 0.930, the OD value of the control well is 0.623, ΔA_{636} = 0.930 - 0.623 = 0.307, and the calculation result is:

$$\text{PTP-1B activity (U/kg wet weight)} = (0.307 - 0.0209) \div 0.0048 \times \frac{0.72}{0.02} \div \frac{0.1}{0.9} \div 1000$$

$$= 19.31 \text{ U/kg wet weight}$$

Detect 10% mouse liver tissue homogenate, 10% mouse brain tissue homogenate, 10% green radish tissue homogenate, and 1×10^6 Jurkat cell, according to the protocol, the result is as follows:



Statement

1. This assay kit is for Research Use Only. We will not response for any arising problems or legal responsibilities causing by using the kit for clinical diagnosis or other purpose.
2. Please read the instructions carefully and adjust the instruments before the experiments. Please follow the instructions strictly during the experiments.
3. Protection methods must be taken by wearing lab coat and latex gloves.
4. If the concentration of substance is not within the detection range exactly, an extra dilution or concentration should be taken for the sample.
5. It is recommended to take a pre-test if your sample is not listed in the instruction book.
6. The experimental results are closely related to the situation of reagents, operations, environment and so on. Elabscience will guarantee the quality of the kits only, and NOT be responsible for the sample consumption caused by using the assay kits. It is better to calculate the possible usage of sample and reserve sufficient samples before use.